

Applicant : Stichting voor de Technische Wetenschappen
Serial No. : 09/762,916
Int. Fil. Date : August 16, 1999
Page 2

C
sequence having four binding sites for LexA from *E. coli*. This sequence is from 5' - 3' :
GTCGACTGCTGTATATAAAACCAGTGGTTATATGTACAGTACTTGTACTGTA
CATATAACCACTGGTTTTATATACAGCAAGCTTGGATCCGTCGAC (SEQ ID NO:1). The
5' side of this sequence comprises a SalI site, the 3' side a HindIII-BamHI-SalI site (all
shown in bold type). Downstream from the LexA binding sites in the HindIII and
BamHI sites, the human heat shock factor-inducible promoter (0.29 kbp HindIII/NcoI
fragment) and the luciferase reporter gene inclusive of SV40 polyadenylation signal
(1.9 kbp NcoI/BamHI fragment) are cloned in a three-way ligation. The human heat
shock factor-inducible promoter (hsp70; accession numbers M59828 and M34267;
nucleotides 52 to 244) can be obtained by means of PCR amplification on human
genomic DNA (Cat. No. 6550-1; Clontech, Palo Alto, USA). As PCR primers, forward
primer 5' - 3' : AAGCTTGGGAGTCGAAACTTCTGGAATATTCGCCAACTTTCAGCCGACG
ACTTATAAAACGCCAGGGGCAAGC (SEQ ID NO:2) may be considered; and as reverse
primer 5' - 3' : CCATGGTTTATAGCTTCCTTAGCTCCTGAAAATCTCGCCAAGCTCCCGG
GGTCCGCGAGAAGAGCTCGGTCCTTCCGG (SEQ ID NO:3). The forward primer
comprises a HindIII site, the reverse primer comprises a NcoI site (given in bold print).
The luciferase reporter gene inclusive of SV40 polyadenylation signals were obtained
through NcoI/BamHI digestion of the pGL3 control vector (Cat. no E1741; Promega,
Madison, USA). In the thus obtained vector, in the HindIII site between the LexA
binding sites and the heat shock promoter, either a 2.1 kbp HindIII fragment of phage
lambda is cloned (Pharmacia Biotech, Uppsala, Sweden), or a 1.7 kbp scs HindIII
fragment. The 1.7 kbp scs DNA fragment is isolated from genomic *Drosophila* DNA
(Cat. #6940-1, Clontech, Palo Alto, USA) with the aid of PCR primers (Forward primer
5' - 3' : GATCAAGCTTATGATCTGCGTATGATACCAAATTTCTG (SEQ ID NO:4);
Reverse primer 5' - 3' : GACAAGCTTACATTGCTGGGCGAGCTGCGCCAATCG (SEQ ID
NO:5)). At the ends of these primers HindIII restriction enzyme sites were located.
The vector with the Lambda fragment (control) is indicated as reporter construct a, the
vector with the scs fragment as reporter construct b. Restriction enzyme digestions,